IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of	:	
Koji TESHIMA et al	:	
Serial No. 10/508,339	:	Group Art Unit 1625
Filed on October 25, 2004	:	Examiner: David K. O'Dell

For: REMEDY FOR SLEEP DISTURBANCE

DECLARATION UNDER 37 CFR §1.132

Honorable Commissioner of
Patents,
P.O. Box 1450
Alexandria, Virginia 22313-1450

Sirs:

I, Koji TESHIMA, citizen of Japan and residing at 5-2-4 Ohzenjihigashi, Asao-ku, Kawasaki-shi, Kanagawa 215-0018, Japan, sincerely declare;

That I am the Senior Research Scientist of Pharmacology Department IV (CNS), Research Division, Mitsubishi Tanabe Pharma Corporation,

That my education and employment history is as follows:

1. I graduated from Department of Pharmacology, Gifu Pharmaceutical University, Japan, Graduate School of Pharmacology, in March 1987,
2. I received a Doctor's degree in Pharmacology from Department of Pharmacology, Faculty of Pharmaceutical Sciences, Kyushu University, Japan, in February 1991,
3. I studied abroad from August 1997 to June 1999 at the Center for Research on Occupational and Environmental Toxicology, Oregon Health Sciences University, as a visiting scientist, and
4. since April 1991 up to this time, I have been an employee of Mitsubishi Pharma Corporation (now Mitsubishi Tanabe Pharma Corporation), and engaged in the research work at the Reseach Laboratories of said Corporation;

That I am a member of Society for Neuroscience (USA), the

Japanese Pharmacological Society (Councilor), and the Japanese Society of Neuropsychopharmacology;

That I am a co-author of the following papers etc.:

1. Miyakawa K, Uchida A, Shiraki T, Teshima K, Takeshima H and Shibata S; ORL1 receptor-mediated down-regulation of mPER2 in the suprachiasmatic nucleus accelerates re-entrainment of the circadian clock following a shift in the environmental light/dark cycle. *Neuropharmacology* 52, 1055-1064, 2007,

2. Kataoka H, Sugahara K, Shimano K, Teshima K, Koyama M, Fukunari A and Chiba K; FTY720, sphingosine 1-phosphate receptor modulator, ameliorates experimental autoimmune encephalomyelitis by inhibition of T cell infiltration. *Cell Mol Immunol*.2, 439-448, 2005,

3. Teshima K and Shibata S; A role of nociceptin in the entrainment mechanism of the biological clock. *Nihon Shinkei Seishin Yakurigaku Zasshi*.25, 203-211, 2005. Review. in Japanese,

4. Teshima K, Minoguchi M, Tounai S, Ashimori A, Eguchi J, Allen CN and Shibata S; Nonphotic entrainment of the circadian body temperature rhythm by the selective ORL1 receptor agonist W-212393 in rats. *Br. J. Pharmacol.* 146, 33-40, 2005,

5. Teshima K, Kim SH; and ALLEN CN; Characterization of an apamin-sensitive potassium current in suprachiasmatic nucleus neurons. *Neuroscience* 120, 65-73, 2003, and

6. Moriya T, Ikeda M, Teshima K, Hara R, Kuriyama K, Yoshioka T, Allen CN and Shibata S; Facilitation of a-amino-3-hydroxy-5-methylisoxazole-4-propinate receptor transmission in the suprachiasmatic nucleus by aniracetam enhances photic responses of the biological clock in rodents. *J. Neurochem.* 85, 978-987, 2003;

That I am one of the inventors of the above-identified U.S. patent application SN 10/508,339; and

That I conducted the following experiments to demonstrate the unexpected superior effect of the compound of the present

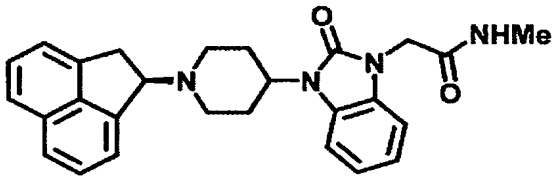
invention (compound of the formula (I) of the above-identified U.S. patent application SN 10/508,339) in that it has a selective and strong affinity for ORL-1 receptor, the results of which follow hereunder.

Experiments

Test compounds

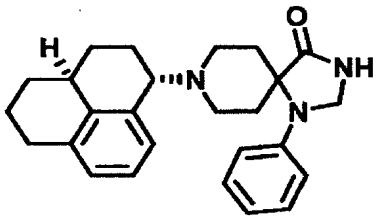
Compound of the present invention

Compound C (compound of Example 18)

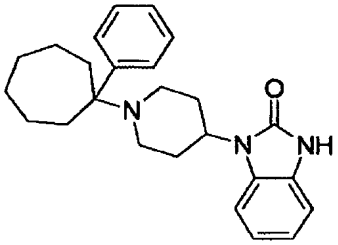


Compound for comparison (compound of cited reference)

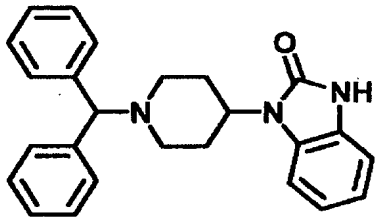
Comparison Compound 1: compound described on page 4940, Fig. 1 of cited reference 1 (Jenck et al, Proceedings of the National Academy of Sciences 2000, 97, 4938-4943)



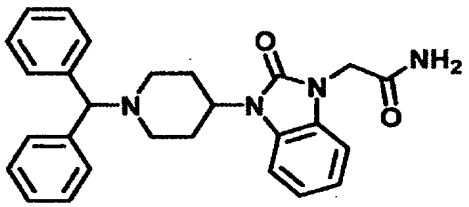
Comparison Compound 2: compound of Example 17 of cited reference 2 (Ito et al, US 6,423,725 (corresponding to WO 9936421)



Comparison Compound 3: compound on page 38, Table 5, first compound of cited reference 3 (Tulshian, D. et al, WO2000/06545)



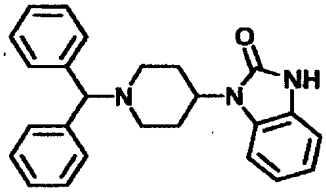
Comparison Compound 4: compound on page 60, the lowermost compound of cited reference 3



Comparison Compound 1 was produced according to the method described in cited reference 1, and Comparison Compound 2 was produced according to the method described in cited reference 2. Comparison Compounds 3 and 4 were produced according to the following method.

Production method of Comparison Compound 3

1-(1-Benzhydryl-piperidin-4-yl)-1,3-dihydrobenzimidazol-2-one



A mixture of 4-(2-oxo-1-benzomidazolyl)-piperidine (5 g, 23 mmol) and triethylamine (8.7 ml, 50 mmol) in dimethylformamide (DMF, 50 ml) was stirred at rt. To this was added α-chloro-diphenylmethane (5 g, 25 mmol) and the mixture was stirred at 100°C for 6 hr. The reaction mixture was poured into water and

the mixture was extracted with ethyl acetate. The extract was washed with water and saturated aqueous ammonium chloride solution, dried over magnesium sulfate, and concentrated. The obtained residue was purified by silica gel column chromatography to give the title compound (3.8 g).

^{1}H-NMR($CDCl_3$).δ_{TMS}: 1.77(m, 2H), 2.05(m, 2H), 2.51(m, 2H), 3.07(d, J=12 Hz, 2H), 4.34((s, 1H), 4.31-4.40(m, 1H), 7.04-7.14(m, 3H), 7.17-7.21(m, 2H), 7.27-7.34(m, 5H), 7.45-7.47(m, 4H), 10.16(s, 1H)

ESI-MS$(M+H)^+$: 384

Production method of Comparison Compound 4

[3-(1-Benzhydryl-piperidin-4-yl)-2-oxo-2,3-dihydro-benzimidazol-1-yl]-acetic acid ethyl ester

(1-Benzhydryl-piperidin-4-yl)-1,3-dihydrobenzimidazol-2-one (2.2 g, 5.7 mmol) was dissolved in dimethylformamide (DMF, 10 ml). Sodium hydride (250 mg, 60%) was added and the suspension was stirred at rt for 30 min. Ethyl bromoacetate (1 g, 6 mmol) was added and the mixture was stirred for 5 hr. The reaction mixture was poured into water and the mixture was extracted with ethyl acetate. The extract was washed with water and saturated aqueous ammonium chloride solution, dried over magnesium sulfate, and concentrated. The obtained residue was purified by silica gel column chromatography to give the title compound (2.2 g).

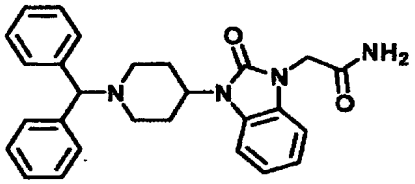
^{1}H-NMR($CDCl_3$).δ_{TMS}: 1.26(t, J=7.2Hz, 3H), 1.77(m, 2H), 2.03(m, 2H), 2.52(m, 2H), 3.06(d, J=12 Hz, 2H), 4.21(q, J=7.2 Hz, 2H), 4.33(s, 1H), 4.29-4.38(m, 1H), 4.60(s, 2H), 6.88(m, 1H), 7.05-7.14(m, 2H), 7.16-7.20(m, 2H), 7.26-7.33(m, 5H), 7.44-7.46(m, 4H)

[3-(1-Benzhydryl-piperidin-4-yl)-2-oxo-2,3-dihydro-benzimidazol-1-yl]-acetic acid

[3-(1-Benzhydryl-piperidin-4-yl)-2-oxo-2,3-dihydro-benzimidazol-1-yl]-acetic acid ethyl ester (2 g) was dissolved in ethanol (5 ml) and 4N-aqueous sodium hydroxide solution (3 ml) was added. The mixture was stirred at rt for 2 hr. The reaction mixture was poured into water, thereto was added to 1N-hydrochloric acid for neutralization, and the mixture was

extracted with chloroform. The extract was washed with water and saturated brine, dried over magnesium sulfate, and concentrated. The obtained solid was washed with ethyl acetate to give the title compound (1.6 g).
^{1}H-NMR (DMSO-d_6)δ_{TMS}: 1.66(m, 2H), 2.00(m, 2H), 2.44(m, 2H), 2.94(d, J=12 Hz, 2H), 3.99(s, 2H), 4.19(m, 1H), 4.39(s, 1H), 6.88(m, 1H), 6.95-7.03(m, 2H), 7.17-7.21(m, 2H), 7.27-7.33(m, 5H), 7.48-7.50(m, 4H)

2-[3-(1-Benzhydryl-piperidin-4-yl)-2-oxo-2,3-dihydro-benzimidazol-1-yl]acetamide



[3-(1-Benzhydryl-piperidin-4-yl)-2-oxo-2,3-dihydro-benzimidazol-1-yl]-acetic acid (1 g, 2.3 mmol) was dissolved in dichloromethane (15 ml). Thionyl chloride (0.5 ml) was added under ice-cooling, and the mixture was stirred at rt for 3 hr. The solvent was evaporated and aqueous ammonia (10 ml) was added to the obtained residue under ice-cooling, and the mixture was further stirred under ice-cooling. The precipitated crystals were collected by filtration to give the title compound (0.34 g).
^{1}H-NMR($CDCl_3$).δ_{TMS}: 1.75(m, 2H), 2.04(m, 2H), 2.50(m, 2H), 3.07(d, J=12 Hz, 2H), 4.33((s, 1H), 4.29-4.38(m, 1H), 4.51(s, 2H), 5.66(s, 1H), 6.20(s, 1H), 7.04-7.07(m, 1H), 7.10-7.21(m, 4H), 7.27-7.35(m, 5H), 7.44-7.47(m, 4H)
ESI-MS$(M+H)^+$: 441

Experimental Method and Measurement

Experiment 1: ORL-1 receptor binding test

Binding test of [^{3}H]-nociceptin was carried out using a standard membranes prepared from human ORL1 receptor expressed HEK293 cells. Specifically, 50 µl of a test substance solution of each concentration, 900 µl of a solution of the receptor

membranes, and 50 μl of a labeled ligand [^{3}H]-nociceptin were added to a polypropylene tube successively, and were subjected to reaction at 25°C for 60 minutes. The reaction buffer (total volume 1 ml) containing 50 mmol/l HEPES, 1 mmol/l EDTA, 10 mmol/l $MgCl_2$, 0.1% BSA with protein inhibitor cocktail, pH7.4 was sucking-filtered with Whatman GF/B, glass filter in a cell harvester. The filter was three times washed with an ice-cooled, 50 mmol/l HEPES buffer solution, and put into a measurement vial. ACS-II (2 ml, Amersham Pharmacia Biotech), liquid scintillation cocktail was added, and then the radioactivity was measured using a liquid scintillation counter (LSC-5100, ALOKA CO., LTD.). Non-specific binding was determined in the presence of J-113397 (J Med Chem, 42, 5061-5063, 1999; 10 μmol/l). Binding inhibition (%) and inhibition constant (Ki value) were calculated according to the following calculation formulae.

Binding inhibition (%) = $\{1-(B-N)/(T-N)\}\times 100$

N: Amount of non-specific binding, T: Amount of total binding, B: Amount of binding in the presence of test substance

Inhibition constant (Ki value) = $IC_{50}/(1+L/Kd)$

IC_{50}: 50% Inhibition concentration, L: Concentration of a labeled ligand, Kd: Dissociation constant of a labeled ligand

Experiment 2: μ receptor binding test

Binding test of [^{3}H]-DAMGO was carried out using a standard membranes prepared from human μ receptor expressed HEK293 cells. Specifically, 50 μl of a test substance solution of each concentration, 900 μl of a solution of the receptor membranes, and 50 μl of a labeled ligand [^{3}H]-DAMGO were added to a polypropylene tube successively, and were subjected to reaction at 25°C for 60 minutes. The reaction solution (total volume 1 ml) containing 50 mmol Tris, 5 mmol/l $MgCl_2$, 0.1% BSA with protein inhibitor cocktail, pH7.4 was sucking-filtered with Whatman GF/B, glass filter in a cell harvester. The filter was three times washed with an ice-cooled, 50 mmol/l Tris buffer solution, and put into a measurement vial. ACS-II (2 ml, Amersham Pharmacia Biotech), liquid scintillation cocktail was added, and then the

radioactivity was measured using a liquid scintillation counter (LSC-5100, ALOKA CO., LTD.). Non-specific binding was determined in the presence of non-labeled DAMGO (10 mol/l). Binding inhibition (%) and inhibition constant (Ki value) were calculated according to the following calculation formulae.

Binding inhibition (%) = $\{1-(B-N)/(T-N)\}\times 100$

N: Amount of non-specific binding, T: Amount of total binding, B: Amount of binding in the presence of test substance

Inhibition constant (Ki value) = $IC_{50}/(1+L/Kd)$

IC_{50}: 50% Inhibition concentration, L: Concentration of a labeled ligand, Kd: Dissociation constant of a labeled ligand

Experimental results

The results of Experiments 1 and 2 are shown in the following Table.

	ORL-1 Ki (nmol/L)	μ Ki (nmol/L)
Compound C (Example 18, present invention)	0.2	76
Comparison Compound 1 (cited reference 1)	0.9	33
Comparison Compound 2 (cited reference 2)	0.22	0.6
Comparison Compound 3 (cited reference 3)	>100 (IC50)	628.8
Comparison Compound 4 (cited reference 3)	>100 (IC50)	482.7

The compound of the present invention showed stronger affinity for ORL-1 receptor and weaker affinity for μ receptor as compared to the compound of the cited reference. Consequently, it has been demonstrated that the compound of the present invention has selective and strong affinity for ORL-1 receptor.

I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these

statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Signed at Yokohama , Japan on this 7th day of December, 2007

Koji Teshima

Koji TESHIMA